SMALL MOLECULE INHIBITORS OF BOTULINUM NEUROTOXINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. Nos. 60/707,531, filed 12 Aug. 2005, and 60/723,442, filed 5 Oct. 2005, both of which are herein incorporated by reference in their entirety.

ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made by employees of the United States Army Medical Research and Materiel Command, which is an agency of the United States Government. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] 1. Field of the Invention

[0004] The present invention relates to compounds which inhibit botulinum neurotoxin. In particular, the present invention relates to compounds which inhibit botulinum neurotoxin serotype A light chain (BoNT/A LC) metalloprotease activity.

[0005] 2. Description of the Related Art

[0006] Botulinum neurotoxins (BoNTs) are produced by spore forming anaerobic bacteria *Clostridium botulinum*, and are among the most lethal of biological poisons (LD_{50}=0.001 μg per Kg). See Schmidt & Stafford (2003) Appl. Environ. Microbiol. 69:297-303; Kessler & Benecke (1997) Neurotoxicology 18:761-770; and Burnett et al. (2005) Nat Rev Drug Discov 4(4):281-297. Seven immunologically distinct BoNT serotypes (designated A-G) have been identified. See Simpson, L. L. (1989) Botulinum Neurotoxin and Tetanus Toxin, Academic Press, New York.

[0007] Exposure to BoNTs, for example, through contaminated food, can result in life threatening flaccid paralysis. See Shapiro, et al. (1998) Ann. Intern. Med. 129:221-228. Furthermore, BoNTs have been weaponized in highly toxic aerosol form, and consequently pose a significant threat to both to civilian and military populations. See Franz, et al. (1997) JAMA 278:399-411; and Amon, et al. (2001) JAMA 285: 1059-1070.

[0008] As indicated, these enzymes have been weaponized in aerosol medium, and airborne release or direct contamination (e.g. foodstuffs) represent significant threats to both military and civilian populations. See Paddle, B M (2003) J Appl Toxicol 23(3):139-170; Clarke, S C (2005) Br J Biomed Sci 62(1):40-46; Hicks et al. (2005) Curr Med Chem 12(6):667-690; and Josko, D (2004) Clin Lab Sci 17(1):30-34. And, with the increased use of BoNTs as therapies for a range of medical conditions and superficial cosmetic purposes, there is the increased potential for accidental overdosing. Furthermore, as the popularity of BoNTs as therapeutics continues to grow, these enzymes are increasingly being manufactures overseas, where less strict controls may allow clandestine organizations to obtain large quantities of these toxins in very concentrated, pure, and easily stored formulations. See Comella & Pullman (2004) Muscle Nerve 29(5):628-644; Gormley et al. (1997) Muscle Nerve Suppl 6:S14-20; Marks, J D (2004) Anesthesiol Clin North America 22(3):509-532, vii; Noonan & Adler (2002) Newsweek 139(19):50-56, 58; O'Brien, C F (2001) Adv Neurol 87:265-269; O'Brien, C F (2002) Clin J Pain 18(6 Suppl):S182-190; O'Brien, D (2003) J Perianesth Nurs 18(2):126-134; Rossetto (2001) Toxicon 39(1):27-41; Shukla & Sharma (2005) Crit. Rev Microbiol 31(1):11-18; and Turton et al. (2002) Trends Biochem Sci 27(11):552-558. As a result, there is an urgent need for therapeutic countermeasures against BoNTs. See Goodnough, et al. (2002) FEBS Lett. 513:163-168.

[0009] BoNT is secreted as a holotoxin composed of two peptide chains that are linked by a disulfide bridge. See Lacy & Stevens (1999) J. Mol. Biol. 291:1091-1104. The heavy chain is responsible for: (1) targeting and binding to surface receptors on nerve terminals; (2) translocation into the neuronal cytosol via the formation of a low pH endosome; and (3) protecting the substrate binding cleft of the light chain prior to neuronal internalization. See Turton, et al. (2002) Trends Biochem. Sci. 27:552-558; and Singh, B. R. (2000) Nat. Struct. Biol. 7 (2000) 617-619. The light chain, which dissociates from the heavy chain in the low endosomal pH, is released into the cytosol where it acts as a zinc metalloprotease that cleaves soluble NSF-attachment protein receptor (SNARE) proteins: synaptosomal-associated protein of 25 kDa (SNAP-25), synaptobrevin, and syntaxin. BoNT serotypes A, C, and E cleave SNAP-25; serotypes B, D, F, and G cleave synaptobrevin; and serotype C can also use syntaxin as substrate. See Binz, et al. (1994) J. Biol. Chem. 269:1617-1620; Schiavo, et al. (1992) Nature 359:832-835; Schiavo, et al. (1993a) J. Biol. Chem. 268:23784-23787; Schiavo, et al. (1993c) J. Biol. Chem. 268:11516-1151915; Schiavo, et al. (1993b) J. Biol. Chem. 269:20213-20216; and Blasi, et al. (1993b) EMBO J. 12:4821-4828. Without functional SNARE complexes, acetylcholine is not released into neuromuscular junctions, thereby leading to paralysis.

[0010] Research to identify peptide and small molecule inhibitors of BoNT serotype A (BoNT/A) has targeted both holotoxin translocation and light chain (BoNT/A LC) metalloprotease activity. Sheridan et al. and Deshpande et al. have shown that a number of antimalarial agents interfere with BoNT/A translocation into nerve cytoplasm. See Sheridan, et al. (1997) Toxicon 35:1439-1451; and Deshpande, et al. (1997) Toxicon 35:433-445.

[0011] Specifically, it has been shown that several antimalarial compounds act subsequent to toxin binding to cell-surface receptors, and it has been hypothesized that these agents inhibit BoNT/A cytosol entry by raising endosomal pH (an endosomal pH of 5.5 or lower is needed for release into the cytoplasm). Hayden et al. have found that BoNT/A LC is inhibited by mM concentrations of known protease inhibitors: captopril, lysinopril, and enalapril. See Hayden, et al. (2003) J. Appl. Toxicol. 23:1-7. In the same study, it was also reported that a number of short peptides, from specific "hinge" libraries, inhibit BoNT/A LC activity by as much as 51% at concentrations as low as 0.5 μM. Using a chromatographic method, Schmidt et al. identified the peptide motif CRATKML as a potent inhibitor. See Schmidt, et al. (1998) FEBS Lett. 435:61-64. In a subsequent study, the Cys residue of CRATKML was replaced with thiol containing organic moieties, and it was found that a 2-mercapto-3-phenylpropi- only containing derivative was the most effective (Ki=0.3 μM). See Schmidt & Stafford (2002) FEBS Lett. 532:423-426.

[0012] Neither the currently available BoNT antitoxin nor antibodies can counter these toxins once they are inside neurons; currently, critical care mechanical ventilation is the only treatment option. However, the effects of internalized BoNTs